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Response of Three South-central Alaskan Spruce Species to Inoculation with Local Blue-stain Isolates

Keith M. Reynolds

U.S.D.A. Forest Service, Pacific Northwest Research Station, Portland, OR 97208. Address of author is Institute of Northern Forestry, 201 East Ninth Avenue, Suite 206, Anchorage, AK 99501.

ABSTRACT

Reynolds, K. M. 1990. Response of three south-central Alaskan spruce species to inoculation with local blue-stain isolates. Plant Disease 00:000-000. A common blue-stain associate of spruce beetles on the Kenai Peninsula (south-central Alaska) was provisionally identified as *Leptographium abietinum*. Recovery rates of blue-stain isolates from stained and non-stained wood were nearly equal when trees were sampled 12 mo after beetle attack, but 92 % of successful isolations came from stained wood when trees were sampled 2 mo after beetle attack. Lutz, Sitka, and white spruce trees were inoculated with isolates of four morphological variants of *L. abietinum*. Morphological differences in isolates were not associated with significant differences in blue-stain incidence either within or among spruce species. Lesion length varied significantly with isolate but not with tree species.

Additional key words: *Leptographium abietinum, Leptographium lundbergii*

Since the mid-1970s, recurring spruce beetle (*Dendroctonus rufipennis* Kirby) outbreaks have caused widespread mortality in spruce stands on the Kenai Peninsula in south-central Alaska (2, 3, 4). Risk and hazard of outbreaks on the Kenai Peninsula depend on forest community type and are highest in certain open-canopied white spruce (*Picea glauca* (Münch) Voss) or Lutz spruce (*P. X lutzii* Little) communities (21). However, damage has also occurred in Sitka spruce (*P. sitchensis* (Bong.) Carr.) and black spruce (*P. mariana* (Mill.) B.S.P.) communities.

At least ten species of blue-stain fungi (Ophiostomataceae) are associated with spruce beetles attacking white spruce and Engelmann spruce (*P. engelmannii* Parry) in North America (6, 8, 9, 11, 14, 22). *Leptographium* anamorphs have been described for *Ophiostoma ips* (Rumb.) Nannf., *O. piceaperdum* (Rumb.) von Arx, and *O. europhioides* (Wright & Cain) Solh., but the latter two species may be synonymous species (11). *Leptographium abietinum* (Peck) Wingfield and *L. engelmannii* Davids., which have no known teleomorphic state and which may also be synonymous species (11), are also associated with spruce beetles. Blue-staining in experimental Lutz spruce trees near Cooper Landing, AK appeared to be primarily associated with at least one *Leptographium* species but species were not identified (20).

This report includes studies conducted in 1988 on isolation and identification of blue-stain fungi from Lutz spruce as well as preliminary pathogenicity tests conducted in 1989. The first objective of the 1988 work was to identify the principal species of blue-stain fungi involved in stain development in previous work (20). A related objective was to determine the incidence of blue-stain species in wood and to estimate incidence of morphological variants within species when possible. The goal of estimating incidence was to obtain initial estimates of the relative importance of

species and their morphological variants with respect to stain development. Preliminary pathogenicity tests were conducted on Lutz, Sitka, and white spruce in 1989 with isolates obtained in 1988 to evaluate variability in responses of Alaskan spruce to isolates as a prelude to more intensive studies of host physiological response to infection by blue-stain fungi in Alaska.

MATERIALS AND METHODS

Collection of blue-stain isolates. Blue-stain isolates were obtained from two sets of Lutz spruce trees near Cooper Landing, AK that had been attacked by spruce beetles following baiting by Hard (10) with the sex attractant frontalin. The first set of 48 trees were attacked in early June 1987 and sampled for blue stain in late May 1988 (12-month sample). The second set of 32 trees were attacked in early June 1988 and sampled for blue stain in mid-August 1988 (2-month sample).

Wood samples for isolation of blue-stain fungi were collected from 3-cm-thick cross-sectional wood disks taken at three bole heights (designated low, middle, and high) in the 12-month samples and at two bole heights (designated middle and high) in the 2-month samples. The lower sampling height was at 137 cm on the bole. Middle and high sampling heights were not identical in the 12- and 2-month samples, but were approximately equivalent and corresponded approximately to mid-points within the lower and middle thirds of crown length, respectively. In general, two wood samples (6.0 X 6.0 X 3.0 cm) were cut from blue-stained areas on each cross-sectional disk. Samples were cut from wood disks such that half of the wood volume contained visible blue-staining. The first sample from a disk was taken from that azimuth on the perimeter at which stain penetration was deepest. If more than 10 % of a disk's perimeter was subtended by stain, a second sample was taken from an area of deep stain penetration that was as nearly opposite to the

perimeter position of the first sample as possible.

Wood samples were stored at -15 C for 2-3 wk prior to isolations. For each wood sample, two chips (1 X 1 X 2 mm) were aseptically excised from a stained and non-stained tangential surface. Stained and non-stained surfaces were selected at radial distances of 5-10 mm within the stain margin (distal to the pith) and beyond the stain margin (proximal to the pith), respectively. Wood chips were plated on commercial malt extract agar and incubated in the dark at 20 C for 4-6 wk.

Identification of isolates obtained in 1988. Following incubation, cultures were sorted into nine groups on the basis of micro- and macroscopic morphological characteristics. Representative isolates of each morphological group were examined by T.C. Harrington (Department of Botany and Plant Pathology, University of New Hampshire) and J. Reid (Department of Botany, University of Manitoba) for species determinations.

Inoculations in 1989. One isolate from each of the four most common morphological groups, representing 84.4 % of all isolates recovered in 1988, were used in inoculation studies in 1989. Circular plugs, 2.5 cm in diameter, of outer and inner bark were punched from bolts cut from the bole of a white spruce tree. Plugs were autoclaved for 30 min at 121 C, inoculated on their inner bark surface with one of the four isolates, and incubated in the dark at 20 C for 7-10 days. Successful inoculation of a bark plug was indicated by abundant mycelial growth on its surface. Visibly contaminated plugs were discarded.

Trees were inoculated during the third week of May 1989. Twenty dominant or codominant trees each of Lutz, Sitka, and white spruce were selected for inoculation in stands near Soldotna, Seward, and Wasilla, AK, respectively. Each of the four isolates was randomly assigned to 5 trees at

each site. Each tree was inoculated at a height of 137 cm on the bole by removing a horizontal row of ten bark plugs and replacing them with inoculated plugs of the assigned isolate. Distance between plugs was 3.5 cm from center to center. Inoculated plugs were stapled in place and covered with 5-cm-wide duct tape to prevent excessive drying.

Tree diameter (cm), age (yr), radial growth increment for the last 5 yr (mm), and crown length (percentage of total tree height) were measured on experimental trees for possible inclusion as covariates in analyses. Diameter, age, and radial growth were measured at a height of 137 cm on the stem.

Assessment of 1989 inoculations and analysis. Inoculations were evaluated during the third week of August 1989. A rectangular area of bark, centered on the row of inoculation plugs and extending 15 cm above and below the inoculation height, was removed from each treated tree and incidence of visible blue-staining in sapwood recorded. If blue stain was not visible on the surface of sapwood, the surface was scraped with a knife to a depth of 1 mm to verify absence of blue stain. Wound reaction to inoculation was measured as vertical extent (cm) of lesion formation above and below an inoculum plug. Area of cambial death was used to delimit extent of wound reaction and was discernible as a depressed area in the 1989 sapwood sheath caused by absence of new wood formation.

Effects of isolate and tree species on mean lesion size (wound reaction) and incidence of visible blue-staining per tree were analyzed by analysis of variance (ANOVA, 23). Prior to ANOVA, possible covariate effects of diameter, age, radial growth increment, and crown length of trees on infection rate and lesion size were analyzed by correlation analysis and visual examination of patterns in scatter plots. For analyses involving stain incidence, the

proportion of inoculum plugs per tree yielding visible blue stain was transformed as the arcsine of the square root of the proportion. The analysis used a full-factorial, complete-block design with respect to the classification variables; the design was unbalanced due to the loss of three experimental trees to spruce beetle attacks. Subsequent to ANOVA, Tukey's Studentized range test (p. 596 in 23) was used to test for significant differences among levels of significant main effects. All significance tests were evaluated at the $\alpha = 0.05$ level.

RESULTS

Species identification for 1988 isolations. Cultures representing the nine morphological groups of isolates were identified as a single *Leptographium* species both by Harrington (*personal communication*) and by Reid (*personal communication*) but some uncertainty remains concerning the species designation. The Lutz spruce isolates are provisionally identified as *L. abietinum* (Peck) Wingfield but may also be *L. lundbergii* Lagerberg & Melin. For purposes of subsequent discussion, isolates used in this study will hereafter be referred to as morphological variants (MVs) of *L. abietinum*.

Recovery of isolates from stained and non-stained wood. *Leptographium abietinum* was recovered from 73.3 % (168 of 229) and 83.3 % (50 of 60) of the 12-month samples and 2-month samples respectively. In the 12-month samples, recovery rates from stained and non-stained wood were about equal in the middle and upper crown sampling positions but at the lower sampling position the recovery rate from non-stained wood was much higher than for stained wood (Table 1). Averaged over the three sampling positions, recovery from stained wood accounted for 45.8 % of all blue-stain isolations in the 12-month sampling. In sharp contrast to results from the 12-month sampling, 92 % (46 of 50) of isolates recovered from the 2-month sampling were from stained wood

(Table 1).

1989 spruce inoculations. Variables considered as possible covariates in subsequent ANOVAs were generally uncorrelated either with stain incidence or with lesion size (Table 2). Examination of scatter plots for stain incidence and lesion size *versus* tree diameter, age, radial growth increment, or crown length also failed to reveal any clear nonlinear relations when plotted observations were identified by either tree species or isolate. Furthermore, it was apparent from scatter plots for stain incidence *versus* radial growth increment that the significant correlation in this instance (Table 2) resulted from three extreme observations. Consequently, no covariates were included in subsequent ANOVA analyses but average tree characteristics were tabulated by tree species (Table 3).

In the ANOVA for stain incidence, the combined effects of tree species, MV, and their interaction only accounted for 29 % of experimental variation and the overall model was not significant. However, the effects of both tree species and MV approached significance given the inclusion of the other terms (Table 4). Preliminary analysis of the data for the ANOVA for lesion length clearly indicated no significant difference in lesion length between symptomatic and nonsymptomatic lesions so observations of mean lesion length per tree were computed without respect to symptom occurrence. The full ANOVA model for lesion length accounted for 35 % of experimental variation and was significant (Table 4). The effects of tree species and the interaction term were clearly nonsignificant whereas the effect of MV was highly significant, primarily due to differences in lesion size associated with MVs 1 and 2 *versus* MV 4 (Table 5).

DISCUSSION

Leptographium abietinum is commonly associated with bark beetle

galleries (15, 18, 24) and has been isolated from spruce beetles in New Hampshire (11). Additional species of blue-stain fungi are associated with spruce beetles in British Columbia (22) and these species may also be associated with spruce beetles in south-central Alaska. Isolation methods used in this study and the limited geographic extent of isolations may have failed to recover species that occurr less frequently in the study area or that occur more commonly in other parts of south-central Alaska. However, the recovery of the single species, provisionally identified as *L. abietinum*, in several hundred isolations is probably indicative of its commoness on the Kenai Peninsula and perhaps in south-central Alaska.

Infection incidence in the inoculation test was probably only slightly greater than visible stain incidence based on the 1988 isolation results (Table 1). Apart from simplifying the assessment procedure, the use of visible symptoms was preferred as a more conservative indicator of successful colonization subsequent to infection because some minimum fungal mass in host tissue is required before blue stain can be visually detected. The effects of tree species and MV in the ANOVA for stain incidence both approached significance but the effect of their interaction was clearly nonsignificant (Table 4), indicating that ranking of MVs with respect to stain incidence was constant across tree species. The tendency toward significance for the effect of tree species was the result of lower mean stain incidence in Sitka spruce (73.9 %) *versus* Lutz (91.9 %) and white spruce (88.4 %). Lower stain incidence associated with MV 4 compared to those for MVs 1, 2, and 3 accounted for the near-significance of MV (Table 5).

In contrast to the ANOVA for stain incidence, the effect of MV in the ANOVA for lesion size was highly significant. The basis for the significance of MV is mostly attributable to the contrast between lesion length associated

with MV 4 *versus* MVs 1 and 2 (Table 5). Although mean responses by MV suggest a positive relation between incidence of blue stain and lesion length (Table 5), the correlation between the two responses based on individual observations was only $r = -0.04$. Effects of tree species and the interaction term were both clearly nonsignificant (Table 4). Mean lesion lengths for Lutz, Sitka and white spruce were 7.27, 7.07, and 7.53, respectively. Paine and Stephen (17), who used similar inoculation methods, reported comparable lesion sizes for infection of loblolly pine (*Pinus taeda* L.) by *Ceratocystis minor* (Hedgcock) Hunt. In contrast to lesion sizes observed in this study, lesions resulting from mechanical wounding of white spruce were 12-15 mm long (13).

Inner bark tissue of inoculum plugs was heavily colonized by isolates during the 7-10 day incubation period following inoculation. Non-colonized plugs remained firm at the end of the incubation period whereas colonized plugs had softened to a spongy consistency indicative of substantial tissue maceration. The inoculum potential of the bark plugs was almost certainly much greater than that of spores introduced by beetles. However, lesions resulting from inoculation of loblolly pine with *C. minor* did not vary significantly with the size of bark plug used as inoculum (17). Thus, although wound response to plugs may be greater than that associated with naturally introduced inoculum, it is probably not proportionately greater.

The lack of a significant difference in lesion size between lesions with and without visible blue stain may be an indication that lesion formation was a response to pre-existing fungal metabolites in bark plugs rather than a result of host reponse to colonization. Limited colonization of sapwood, not accompanied by blue staining, could be an alternative explanation for the lack of difference in wound response (17) but this seems unlikely considering the low frequency of blue-stain isolations from non-stained wood after 2 mo (Table

1). In general, lesions formed in response to autoclaved blue-stain inoculum are significantly smaller than lesions formed in response to non-autoclaved inoculum (17) and are comparable to lesion sizes resulting from mechanical wounding (7, 19). Most enzymes and many toxins involved in eliciting wound response are destroyed by heat sterilization (1). Consequently, inferences based on comparisons between response to autoclaved and non-autoclaved fungal inocula are probably not useful in this instance.

Leptographium abietinum is non-pathogenic or only weakly pathogenic to ponderosa pine (*P. ponderosa* Laws.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings (12) and only weakly pathogenic to lodgepole pine (*P. contorta* Dougl.) (5). Results from this study indicate that local isolates of *L. abietinum* are at least weakly pathogenic to Lutz, Sitka, and white spruce in south-central Alaska. Although the inoculum potential of a single plug is probably greater than that associated with spores introduced during excavation of egg galleries, ten experimental inoculations per tree is only a small fraction of the number of natural inoculations resulting from mass attack by spruce beetles (16). Additional, more detailed studies of the physiological response of Alaskan spruce to infection by *L. abietinum* are therefore necessary to better understand the role that blue stain plays in killing spruce in Alaska.

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Table 1. Recovery of blue-stain isolates from Lutz spruce wood at 3 and 12 months after spruce beetle attack.

Time since attack (mo)	Sample position[b]	Sample size	Isolates recovered[a]	
			Stain	Non-stain
3	Low	-	-	-
	Middle	39	28	3
	High	21	18	1
12	Low	85	16	28
	Middle	89	44	40
	High	55	17	23

[a] Isolations were made at radial positions 5-10 mm within and beyond the limits of visible blue-stain penetration. Two isolations were made at each radial position in each wood sample. In general, isolations were made from two wood samples at each vertical bole position on a tree.

[b] Wood samples for isolations were taken at a height of 137 cm on the bole (low position) and at mid-points of the lower and middle thirds of crown length (middle and upper positions, respectively).

Table 2. Correlations of tree covariates with infection frequency and lesion length for spruce species infected by *Leptographium abietinum*.

Covariate[b]	Correlation with[a] Stain[c]	Lesion length
Diameter	0.19	0.02
Age	-0.11	0.02
5-yr radial growth	0.30 *	0.12
Crown length	0.10	-0.11

[a]* indicates correlation was significant at 0.05 level.

[b]Diameter, age, and radial growth were measured at height = 137 cm. Crown length was expressed as percentage of total tree height.

[c]Proportion of symptomatic inoculations per tree was transformed by using the arc-sine of the square root of the proportion.

Table 3. Means and standard deviations of tree covariates by spruce species.[a]

Species	Diameter (cm)	Age (yr)	Radial growth (mm/5 yr)	Crown length (%)
Lutz spruce	31.1 (6.4)	96.1 (27.8)	8.0 (2.9)	80.9 (13.3)
Sitka spruce	34.0 (5.4)	153.1 (16.1)	6.4 (2.3)	61.3 (10.8)
white spruce	28.5 (2.8)	136.8 (37.4)	7.2 (1.5)	76.0 (11.4)

[a]Diameter, age, and radial growth were measured at height = 137 cm. Crown length was expressed as percentage of total tree height.

Table 4. Analysis of variance for effects of tree species and morphological variant (MV) of *Leptographium abietinum* on lesion size and incidence of visible staining[a].

Model Source	Degrees of freedom	Mean square[b]	P[c]
Stain			
Full model	11	0.20	0.11
Tree species	2	0.35	0.07
MV	3	0.31	0.06
T x MV[d]	6	0.10	0.56
Error	45	0.12	
Lesion size			
Full model	11	2.51	0.03
Tree species	2	1.16	0.36
MV	3	6.00	<0.01
T x MV[d]	6	1.27	0.36
Error	45	1.12	

Table 4. Analysis of variance for effects of tree species and morphological variant (MV) of *Leptographium abietinum* on lesion size and incidence of visible staining[a].

[a]For analysis of variance, percentage of symptomatic inoculations per tree was transformed by using the arc-sine of the square root of the proportion.

[b]The mean square for individual effects was computed from the type III sum of squares, sometimes referred to as the partial sums of squares (p. 588 in 23). Type III sums of squares do not depend on ordering of effects in the model.

[c]Probability of exceeding the associated F-statistic for the mean square, given the degrees of freedom for the effect and error.

[d]Interaction effect of tree species and morphological variant.

Table 5. Frequency of morphological variants of *Leptographium abietinum* isolated from Lutz spruce trees in 1988, and mean lesion length and incidence of visible staining per tree in 1989 using the four most commonly isolated variants obtained in 1988.[a]

Culture type	Frequency (%)	Visible staining[b] (%)	Lesion length[c] (cm)
1	55.5	92.8 A	7.86 A
2	17.0	91.4 A	7.90 A
3	6.9	85.7 A	6.92 AB
4	5.0	67.0 A	6.62 B
5-9	15.6	NA	NA

Table 5. Frequency of morphological variants of *Leptographium abietinum* isolated from Lutz spruce trees in 1988, and mean lesion length and incidence of visible staining per tree in 1989 using the four most commonly isolated variants obtained in 1988[a].

NA = not applicable (types 5-9 were not evaluated).

[a]In May 1989, 20 Lutz, Sitka, and white spruce trees were inoculated at Soldotna, Seward, and Wasilla, AK, respectively. At each site, five trees each were inoculated with ten bark plugs colonized by one of four morphological variants of *Leptographium abietinum*. Means for infection and lesion length followed by the same letter were not significantly different based on Tukey's (p. 596 in 23) studentized range test.

[b]Inoculum plugs yielding visible symptoms of blue stain in sapwood. For analysis of variance, percentage of symptomatic inoculations per tree was transformed by using the arc-sine square root of the proportion. Minimum significant difference in transformed data for Tukey's test = 0.35 radians.

[c]Minimum significant difference for Tukey's test = 1.06 cm.